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14. ABSTRACT

Histone demethylases are a newly discovered class of non-heme iron enzymes that play an important role in regulating transcription and epigenetic inheritance. We have successfully expressed and purified highly active histone demethylases (HDMs), including the cancer-relevant JMJD2C (GASC1). A detailed enzyme kinetic and inhibition analysis of these HDMs was achieved through a range of fluorescence assays, mass spectrometry, and oxygen consumption measurements. An interesting case of cosubstrate inhibition is observed for these HDMs. We have also started to develop specific inhibitors for these enzymes using an enzyme-templated approach that takes advantage of the enzyme's substrate specificity. The identified inhibitors will be tested for the inhibition of JMJD2C in MCF7 breast cancer cells. The developed specific inhibitors could lead to novel breast cancer therapeutics and can also be used as tools for studying the role of histone demethylases in breast cancer cell proliferation.

15. SUBJECT TERMS
Epigenetics, histone demethylases, inhibitor design, GASC1 oncogene

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Introduction

Covalent modification of chromatin by histone methylation has wide-ranging effects on nuclear functions, such as transcriptional regulation, genome integrity, and epigenetic inheritance. [1] Until recently, histone methylation was considered a static modification, but the identification of histone demethylase (HDM) enzymes has revealed that this mark is dynamically controlled.[2,3] Most of the identified histone demethylases are α-ketoglutarate (α-KG) dependent, O_2-activating non-heme iron enzymes (Figure 1) that catalyze the lysine demethylation through a hydroxylation reaction (Scheme 1).[4]

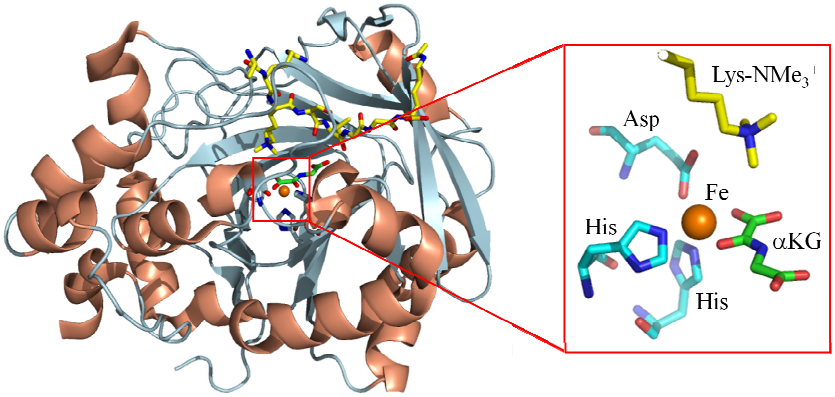
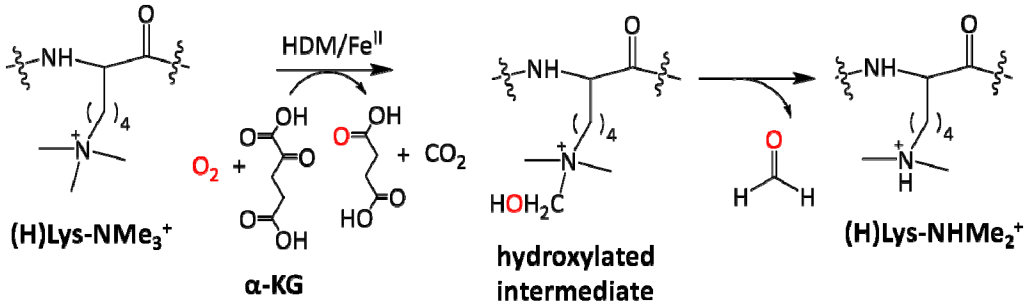
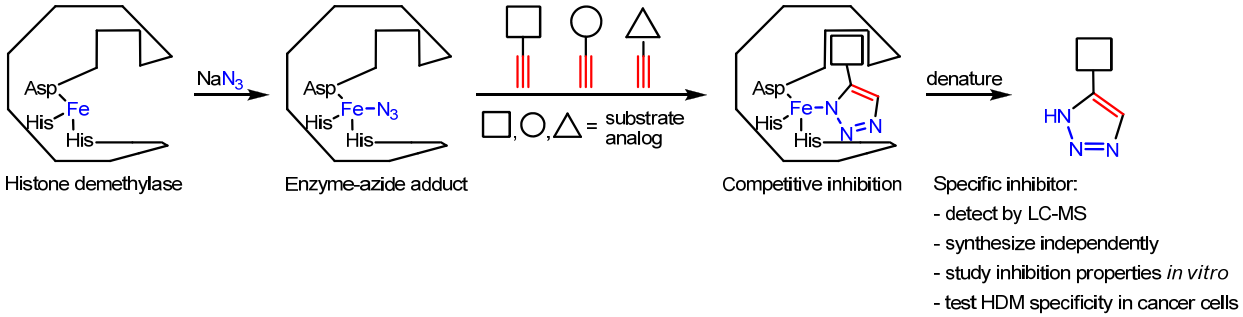


Figure 1. Crystal structure of JMJD2A, a JmjC-domain histone demethylase (HDM).[4]



Scheme 1. Histone demethylation reaction catalyzed by HDMs.

It has recently been shown that the histone demethylase GASC1 (or JMJD2C) is overexpressed in several cancer cells and may be linked to the stem cell phenotypes in breast cancer.[5] In addition, the histone demethylase PLU-1 plays an important role in the proliferation of breast cancer cells through transcriptional repression of tumor suppressor genes.[6] Thus, the development of specific inhibitors for HDMs could provide new avenues for breast cancer therapeutic development. Our approach will employ the design of specific inhibitors of histone demethylases using a novel enzyme-templated Huisgen 1,3-dipolar cycloaddition reaction between the azide adduct of the targeted enzyme and an alkyne substrate analog (Scheme 2). The cycloaddition reaction should proceed at a faster rate than in solution, due to the sequestering of the two components in the enzyme active site. Thus, the enzyme itself, serving as the reaction vessel, is synthesizing its highest-affinity inhibitor. The synthesized compounds will mimic two substrates (O_2 and the methylated amine substrate) and most likely be specific for the targeted enzyme.



Scheme 2. Enzyme-templated approach for development of specific competitive inhibitors.

Body

Task 2a: *HDM expression and purification*. Truncated constructs of JMJD2A,[4] JMJD2E,[4] and JMJD2C[7] containing the JmjN- and JmjC-domains were transformed into the *E. coli* Rosetta II strain and expressed and purified using published procedures.[4,7] Purification was achieved via Ni-NTA superflow and anion-exchange column chromatography to yield enzymes with >90% purity and expression yields of ~5 mg/L for JMJD2A and JMJD2C and ~20 mg/L for JMJD2E. Unfortunately we were not able to successfully express and purify the PLU-1 histone demethylase.

Task 3a: *Fluorescence coupled assay*. Using a modified coupled assay,[8] the HDM activity was monitored via the production of fluorescent NADH formed during formaldehyde oxidation by a formaldehyde dehydrogenase coupled reaction (Figure 2). In all enzymatic assays, an octapeptide (ARK(me_3)STGGK) corresponding to the histone sequence around the methylated lysine was employed as the substrate analog.

Oxygen consumption assay. We have developed for the first time a continuous assay that directly monitors the HDM activity in real time by measuring the dioxygen consumption using a Clark oxygen electrode. This allowed us to obtain a detailed characterization of the enzymatic kinetic properties of the three HMDs investigated (see below).

MALDI-TOF assay. Demethylation of the peptide substrate was also confirmed by mass spectrometry (MALDI-TOF). The amount of the mono- and di- methylated peptide was determined at the end of the reaction, converted to percent conversion of peptide substrate and used to correlate the extent of demethylation with the other enzymatic assays.

Using the developed NADH fluorescence coupled assay, we obtained the kinetic parameters for JMJD2A and JMJD2E that are similar to those described in literature.[8] However, our expressed enzymes display markedly higher activity (Table 1). In addition, we have also performed for the first time the kinetic analysis of the cancer-relevant JMJD2C (GASC1). Important to note that JMJD2E is the most active of the three HMDs and it has been employed in all the initial inhibitor screening studies to allow for a rapid identification of potent inhibitors.

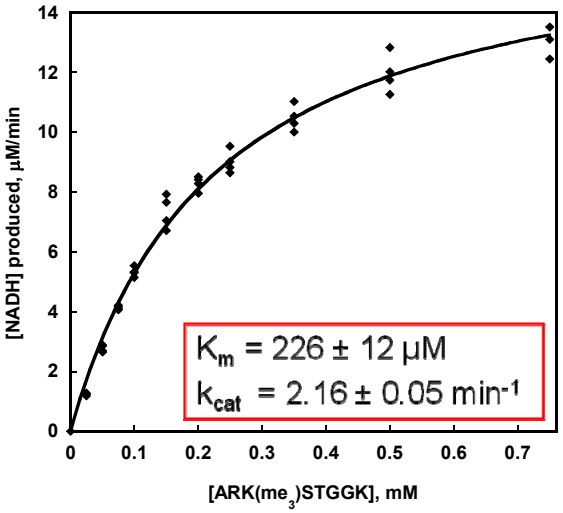


Figure 2. Michaelis-Menten plot of JMJD2E obtained by the fluorescence coupled assay. Reaction conditions: 20 mM Tris pH 7.4, 500 µM α-KG, 500 µM ascorbate, 50 µM $Fe(II)SO_4 \cdot 6H_2O$, 50 mM NaCl, 0.04 units FDH, 2 mM NAD^+, 8 µM JMJD2A/C/E; varying ARK(me_3)STGGK peptide concentrations.

Table 1. Kinetic parameters for the three investigated HMDs.

	JMJD2A	**JMJD2C**	**JMJD2E**
$\mathbf{K_m}$ (µM)	102±11	76±6	226±12
$\mathbf{k_{cat}}$ (min^{-1})	0.14±0.01	0.25±0.01	2.16±0.05

Task 3b: The observed high activity of the HMDs allows us to monitor the enzymatic reaction in real-time using an oxygen electrode (Figure 3) and we found that the K_m of ARK(me_3)STGGK with JMJD2E is dramatically lower than that obtained from the fluorescence coupled assay. Such a result suggests that the coupled assay leads to artificially increased K_m values and the oxygen consumption assays provide a better kinetic evaluation method. Variation of dioxygen concentration with all other reaction components held constant allowed us to obtain the kinetic parameters of O_2 for JMJD2E (Figure 3) and JMJD2A (K_m = 63 ± 10 µM).

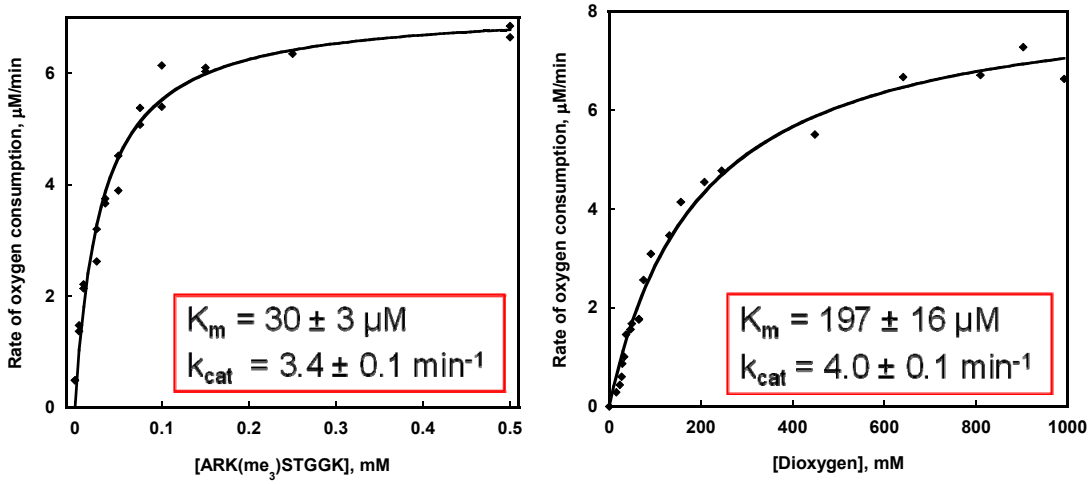


Figure 3. Michaelis-Menten plot for JMJD2E obtained using a Clark oxygen electrode and varying peptide concentration (left) or oxygen concentration (right). Reaction conditions: 20 mM Tris pH 7.4, 300 μM α-KG, 500 μM ascorbate, 2 μM $Fe(II)SO_4 \cdot 6H_2O$, 2 μM JMJD2A/E, and varying peptide or oxygen concentrations, respectively.

While studying the kinetics of α-KG by the fluorescence coupled assay, we observed an inhibitory effect at high α-KG (>1 mM) concentrations (Figure 4), which was confirmed by MALDI-TOF and oxygen consumption assays *(*data not shown*)*. We also observed that α-KG inhibition is more pronounced at low vs. high O_2 concentrations, suggesting a competition between the two species in solution (Table 2).

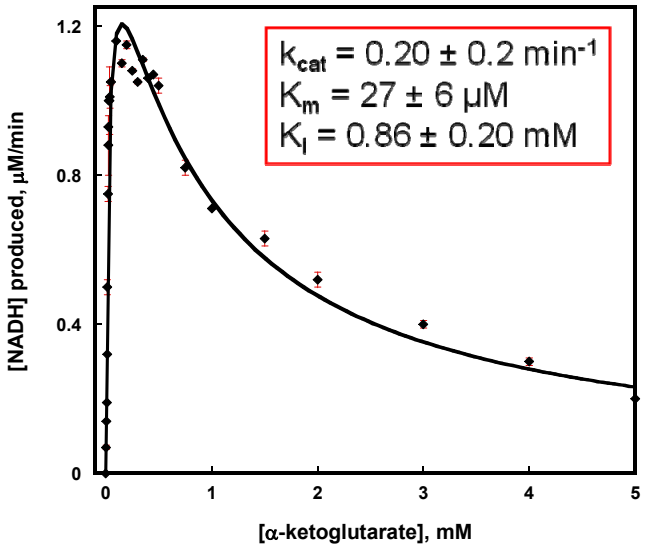


Figure 4. JMJD2A inhibition by α-KG, measured by the fluorescence coupled assay.

Table 2. JMJD2E inhibition by αKG at various O_2 concentrations.

	120 μM O_2	**258 μM O_2**	**980 μM O_2**
K_m (μM)	26 ± 5	25 ± 5	28 ± 6
K_i (mM)	0.9 ± 0.2	1.6 ± 0.3	2.7 ± 0.4

Novel finding: *Relevance of α-KG concentration variation in cancer cells.* Recent reports have implicated mutated isocitrate dehydrogenase enzymes 1 and 2 (*IDH1* and *IDH2*) in multiple types of cancers that are responsible for the interconversion of isocitrate to α-KG.[9] The most common mutations result in a hyperactivity catalyzing the reduction of α-KG to D-2-hydroxyglutarate (2HG), leading to lower concentrations of α-KG in malignant cells.[10] It has also been proposed that 2HG could interfere with the activity of α-KG-dependent enzymes implicated in oncogenesis. Based on our α-KG substrate inhibition results, we speculate that a decrease in αKG concentration could lead to a higher activity of HMDs and an increased histone demethylation and thus an altered gene transcription profile. We plan to further study the implication of the observed α-KG substrate inhibition for HMDs and the effect of α-KG concentration variation in cancer cells.

Task 1b and 2b: *Inhibitor synthesis and evaluation.* We have began our inhibitor development studies by synthesizing a large number of substrate analogs bearing either di- and tri-methylammonium groups or α-keto-acid fragments (Chart 1) to be tested for their inhibition properties. A representative synthetic scheme is shown in Scheme 1 for compound (2b), 2-oxo-2-(propynylamino) acetic acid.

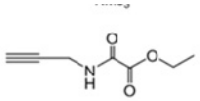
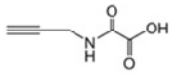
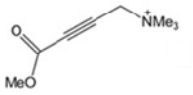
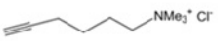
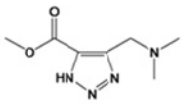
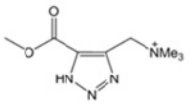
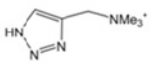
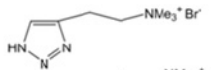
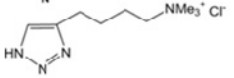
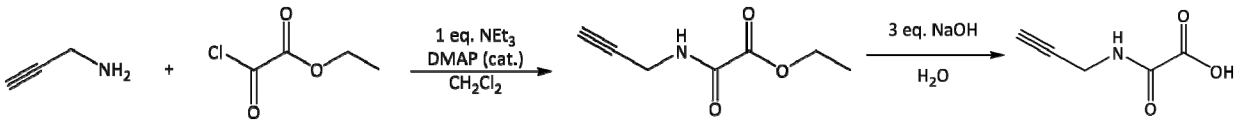
Compound	Structure
(2a) ethyl 2-oxo-2-(prop-2-ynylamino) acetate	
(2b) 2-oxo-2-(propynylamino) acetic acid	
(3) dimethyl ammonium alkyne ester	
(4) trimethyl ammonium alkyne ester	
(5) trimethyl ammonium propyne	
(6) trimethyl ammonium butyne	
(7) trimethyl ammonium hexyne	
(8) methyl 4-((dimethylamino)methyl)-1H-1,2,3-triazole-5-carboxylate	
(9) 1-(5-(methoxycarbonyl)-1H-1,2,3-triazol-4-yl)-N,N,N-trimethylmethanaminium	
(10) *N,N,N*-trimethyl-1-(1*H*-1,2,3-triazol-4-yl)methanaminium	
(11) *N,N,N*-trimethyl-2-(1*H*-1,2,3-triazol-4-yl)ethanaminium	
(12) *N,N,N*-trimethyl-4-(1*H*-1,2,3-triazol-4yl)butan-1-aminium	

Chart 1. List of synthesized substrate analogs for HDM inhibition studies.



Scheme 1. Synthesis of an *N*-oxalylglycine (NOG) alkyne analog, PgAmideAcid.

Task 3b: The synthesized substrate analogs were then tested for their inhibition properties against the three HMDs. The inhibitory properties of our compounds were determined using Dixon plots (Figure 6) to obtain the apparent inhibition constants, K_i's. We use K_i rather than

IC_{50} values as K_i is an absolute value and IC_{50} is substrate- and enzyme-concentration dependent. While the analogs containing trimethylammonium groups show a mild inhibition of JMJD2A and JMJD2E (Table 3), the αKG analogs such as *N*-oxalylglycine (NOG) are good yet unspecific inhibitors of HDMs. By contrast, the designed alkyne NOG analog (PgAmideAcid) shows good affinity for JMJD2E and this compound was used in the enzyme-templated cycloaddition reaction (see below).

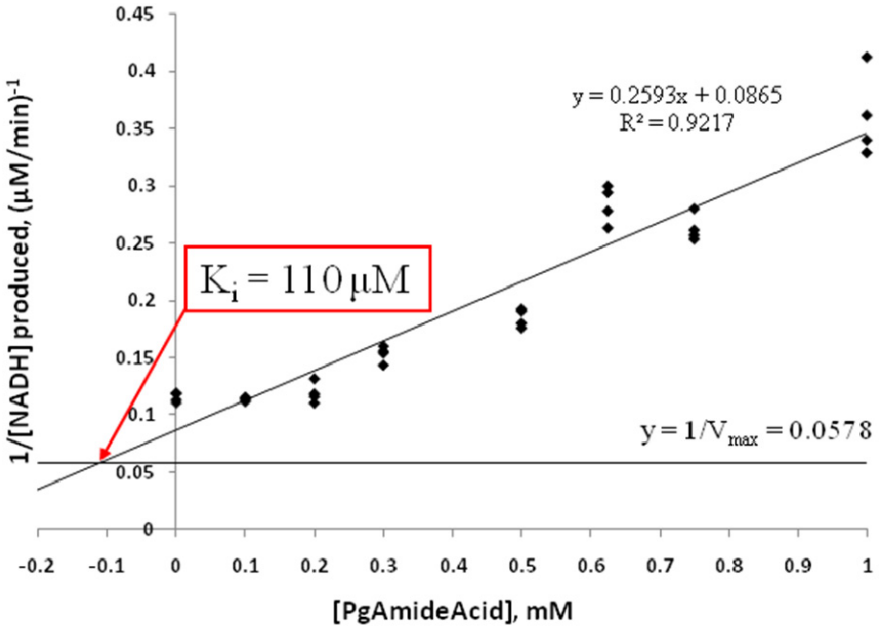
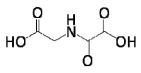
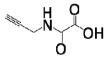
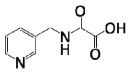
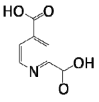
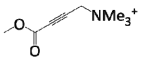
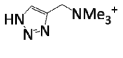


Figure 6. Dixon plot of JMD2E inhibition by PgAmideAcid.

Table 3. Summary of inhibition data for the most promising compounds.

Inhibitor	Structure	Ki (apparent)
N-oxalylglycine		208µM
Propargyl AmideAcid		110µM
Pyridine AmideAcid		215µM
2,4-Pyridine dicarboxylic acid		6µM
NMe_3^+ OMe alkyne		571µM
NMe_3^+ Triazole		1.18mM

Task 2c,d: *Enzyme-templated cycloaddition reaction.* The alkyne NOG analog PgAmideAcid that shows affinity for the HMDs was employed in the enzyme-templated cycloaddition studies.

Preliminary results suggest that in presence of JMJD2E, PgAmideAcid reacts with azide to form the corresponding triazole product, as observed by ESI-MS (Figure 7). This promising result takes advantage of the enzyme's ability to bind both substrate analogs and promote the cycloaddition reaction. We are currently synthesizing the identified triazole product in larger quantities using organic synthesis and will test its inhibitory properties against HMDs.

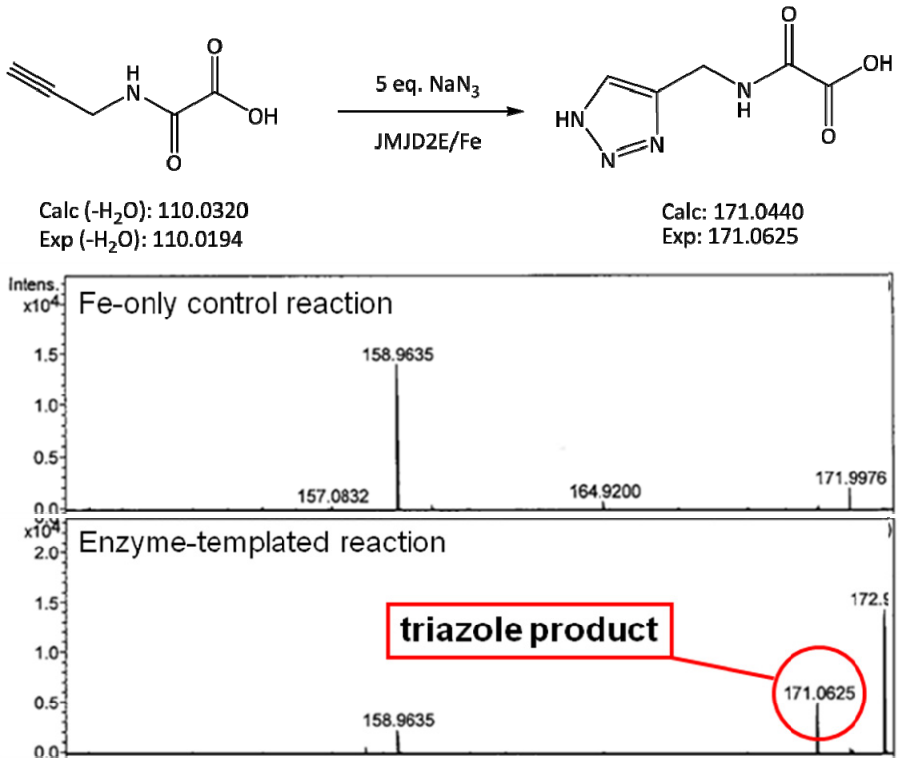


Figure 7. The observed enzyme-templated cycloaddition reaction and ESI-MS evidence for product formation.

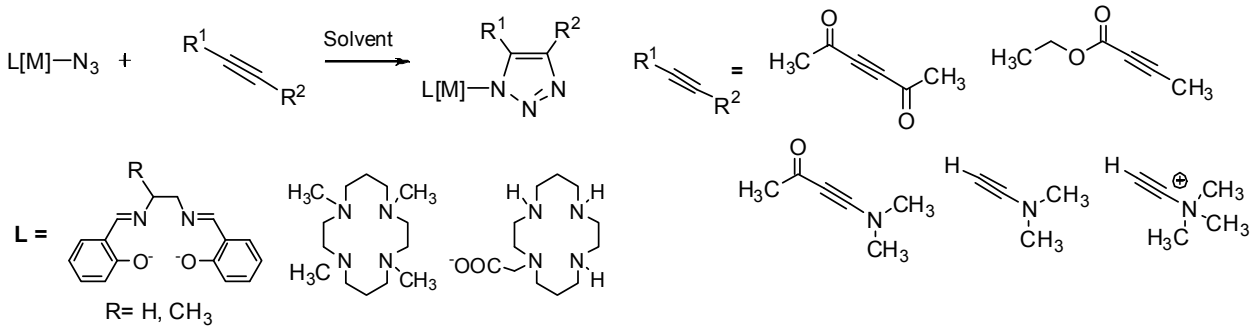
Task 1a-d: Concurently with the enzyme kinetic and inhibition studies and the enzyme-templated cycloaddition reaction studies, we have also investigated the 1,3-dipolar cycloaddition reaction between metal-azide complexes and alkyne reagents, i.e. an inorganic variant of the extensively used "click reaction". In this context, we have synthesized small molecule models of the metal active sites of HDMs and investigated the cycloaddition reaction between the azide adducts of these complexes and various alkynes to determine the scope of the reaction with respect to the alkyne and metal compound properties. The reaction between the azide complexes of bioinorganic metals (e.g. Fe, Co, Ni) that are models of metalloenzymes and alkyne reagents is envisioned as a potential new method of developing triazole-based specific inhibitors for HMDs in particular, and for other O_2-activating metalloenzymes in general.

The synthesis and characterization of six Fe, Co, and Ni mono-azide complexes has been accomplished using salen- and cyclam-type ligands. Then the scope of the inorganic azide-alkyne "click reaction" was investigated using the electron deficient alkyne dimethyl acetylenedicarboxylate. Of the six metal complexes investigated, only the Co and Ni azide complex of the tetra-methylcyclam ligand showed a successful cycloaddition reaction and formation of the corresponding metal-triazolate products that were crystallographically

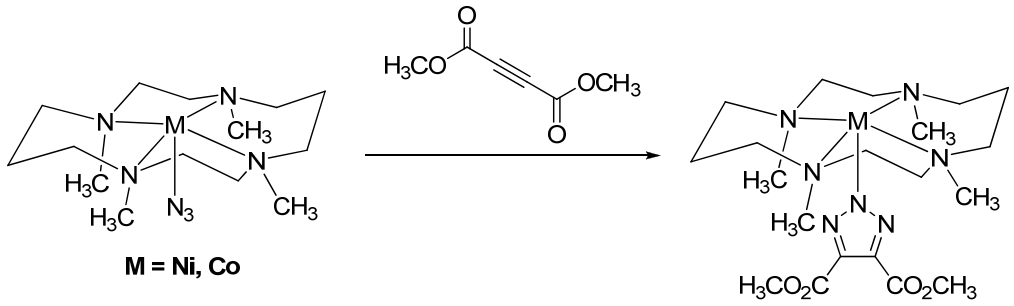
characterized. Moreover, use of a large number of synthesized alkynes that did not yield any cycloaddition reaction product (data not shown), suggesting that an electron deficient is required for the cycloaddition reaction to occur for an inorganic complex. However, the enzyme-templated cycloaddition reaction is expected to occur for a large number of alkyne substrates (see above) due to the enzyme's ability to sequester the two substrates and promote the triazole formation.

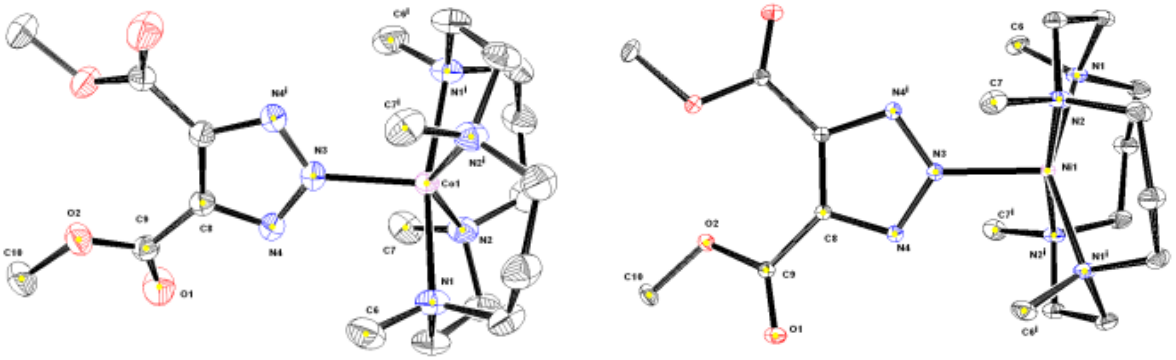
Overall, these results reveal that the success of the cycloaddition reaction between a metal-azide complex and an alkyne substrate is dependent both on the ligand and metal oxidation state of the metal complex, and the electron deficient nature of the alkyne employed. We plan to use the azide complexes that show efficient triazole formation as a method of synthesizing larger quantities of the triazoles that show promising inhibitory properties (**Task 3d**).

Scheme 2. The scope of the studied cycloaddition reaction between metal-azide complexes and various alkynes for the synthesis of metal-triazolate complexes.



Scheme 3. Successful cycloaddition reaction between metal-azide complexes and dimethyl acetylenedicarboxylate and crystal structure of the Co- and Ni-triazolate complexes.





Future Directions

Since only one graduate student has worked on the project for the past year, a one-year no-cost extension was requested and awarded for the proposed work. During this period (August 2011 – July 2012) the remaining tasks will be completed.

Task 3d: We are currently investigating the specificity of the most potent inhibitors versus the various targeted HMDs. In addition, we are continuing to expand the library of substrate analogs and are testing their inhibitory properties.

Task 2e: We plan to also use the azide complexes that show efficient triazole formation as a method of synthesizing larger quantities of the triazoles that show promising inhibitory properties.

Task 2c: We will further study the scope the enzyme-templated cycloaddition reaction to provide us with lead compounds for inhibitor development. Based on the results obtained from the small molecule mimics studies (**Task 1d**), we will employ Co- and Ni-reconstituted HDMs for the enzyme-templated synthesis of triazole compounds in an attempt to detect inhibitors of various efficacy and specificity.

Task 4: The most potent inhibitors will be tested in cancer cell lines. We will use HCC1954 breast cancer cells (that show overexpression of GASC1 histone demethylase) and the developed inhibitors that are specific for GASC1. Test the role of GASC1 in cancer cells by comparing the observed effects when treating with histone demethylase inhibitors the HCC1954 cells vs. MCF10A cells (that do not overexpress GASC1). In addition, we will also employ MCF7 breast cancer cells that show overexpression of PLU-1 histone demethylase, and compare the inhibitory effect versus that in HCC1954 cells and assess the *in vivo* specificity of these inhibitors.

Key research accomplishments

- Expressed and purified three highly active histone demethylases (HDMs).
- Developed a continuous oxygen consumption assay for monitoring the activity of HDMs in real time.
- Obtained a detailed kinetic characterization of the targeted HMDs.
- Discovered a unique case of cosubstrate inhibition of HDMs, which could be directly relevant to the HDM activity in cancer cells.
- Developed a series of substrate analogs that exhibit HDM inhibition properties.
- Provide the proof of concept for the targeted enzyme-templated cycloaddition reaction for the development of specific HDM inhibitors.

Reportable Outcomes

Meeting Abstract

Barbara S. Gordon and Liviu M. Mirica,* "Development of Specific Inhibitors for Histone Demethylases", poster presentation, Era of HOPE conference, DOD CMDRP program, August 2-5, Orlando Florida.

Manuscripts in preparation

Emi Evangelio, Nigam P. Rath, and Liviu M. Mirica,* "Click chemistry between metal-bioinorganic complexes and alkynesubstrates: New methodology for the in-situ synthesis of metalloenzyme-inhibitors."

Barbara S. Gordon and Liviu M. Mirica,* "α-Ketoglutarate substrate inhibition of histone demethylases: Implications for gene transcription regulation in cancer cells."

Conclusion

Histone demethylases are a newly discovered class of non-heme iron enzymes that play an important role in regulating transcription and epigenetic inheritance. We have successfully expressed and purified highly active histone demethylases (HDMs), including the cancer-relevant JMJD2C (GASC1). A detailed enzyme kinetic and inhibition analysis of these HDMs was achieved through a range of fluorescence assays, mass spectrometry and oxygen consumption measurements. An interesting case of cosubstrate inhibition is observed for these HDMs. We have also provided evidence for a novel enzyme-templated synthetic approach that takes advantage of the enzyme's substrate specificity to develop specific inhibitors for these enzymes. The developed specific inhibitors could lead to novel breast cancer therapeutics and can also be used as tools for studying the role of histone demethylases in breast cancer cell proliferation.

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Appendices

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EDUCATION

1999–2005 Ph.D., Chemistry, **Stanford University**, Stanford, CA
Thesis title: "Mechanistic Investigations of Model Complexes Relevant to Copper-Containing Enzymes." Graduate Advisor: *Professor T. Daniel P. Stack*

1996–1999 B.S., Chemistry, **California Institute of Technology**, Pasadena, CA
Undergraduate Research Advisor: *Professor Harry B. Gray*

PROFESSIONAL POSITIONS

2008-present **Assistant Professor**, Department of Chemistry, Washington University in St. Louis

2005-2008 **NIH Postdoctoral Fellow**, University of California, Berkeley
Postdoctoral Advisor: *Professor Judith P. Klinman*

AWARDS AND HONORS

- Ralph E. Powe Junior Faculty Award, Oak Ridge Associated Universities, 2010-2011
- NIH–NRSA Postdoctoral Fellowship, 2007-2008
- Young Investigator Award, Division of Inorganic Chemistry, ACS, 2006
- Franklin Veatch Memorial Fellowship, Stanford University, 2004-2005
- Stanford Graduate Fellowship, Stanford University, 1999-2003
- Taube Prize, Stanford University, 1999
- Merck Index Award for Excellence in Chemistry, California Institute of Technology, 1999
- Carnation Merit Award, California Institute of Technology, 1997-1998
- Silver Medal, International Chemistry Olympiad, Beijing, China, 1995
- Gold Medal, International Chemistry Olympiad, Oslo, Norway, 1994

RESEARCH INTERESTS

Bioinorganic projects include: a) the development of specific inhibitors for non-heme iron histone demethylases, a new class of enzymes that play an important role in transcription regulation; and b) study the role of transition metal ions in amyloid β (Aβ) peptide aggregation in Alzheimer's Disease (AD) and develop modulators of Aβ peptide aggregation using a novel bifunctional strategy that could provide improved strategies for the prevention, diagnosis, and treatment of AD.

Design, synthesis, and characterization of metal complexes as potential catalysts for renewable energy applications. Targeted reactions include: a) the oxidative oligomerization of hydrocarbons catalyzed by Pd^{II}/Pd^{III} complexes, relevant to the conversion of natural gas to liquid fuels; b) the oxidation of water to dioxygen using bimetallic first row transition metal complexes, in the context of solar energy conversion and storage; and c) the electroreduction of CO_2 using low-valent Pd and Ni catalysts, aimed at the conversion of carbon dioxide into useful chemicals.

RESEARCH SUPPORT

- American Chemical Society, Petroleum Research Fund, "Study of Water Oxidation by Binuclear Metal Complexes", October 2009 – August 2011, PI: Mirica
- Department of Defense, Breast Cancer Research Program, "Specific Inhibitors of Histone Demethylases: Novel Chemical Agents for Breast Cancer Therapy", August 2010 – July 2012 (one year no-cost extension), PI: Mirica
- Alzheimer's Disease Research Center Pilot Research Grant, National Institute of Aging – NIH, "Novel Bifunctional Metal Chelators as Selective Binders to Soluble Aβ Oligomers", May 2011 – April 2012, PI: Mirica.
- Department of Energy, Office of Basic Sciences, Catalysis Science Program, "Novel Palladium Catalysts for the Aerobic Oxidative Oligomerization of Methane & Carbon Dioxide Reduction", September 2011 – August 2014, PI: Mirica.

PUBLICATIONS

19. Verma P., Weir J., Mirica L. M., Stack T. D. P.* "Tale of a Twist: Magnetic and Optical Switching in Copper(II) Semiquinone Complexes" *Inorg. Chem.*, **2011**, *50*, DOI: 10.1021/ic200958g.
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16. Khusnutdinova J., Rath N., Mirica L. M.* "Stable Mononuclear Organometallic Pd(III) Complexes and Their C-C Bond Formation Reactivity" *J. Am. Chem. Soc.*, **2010**, *132*, 7303-7305. Featured as "News of the Week" in *Chem. & Eng. News*, **2010**, *88*, 21, 9.
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7. Yoon, J.; Mirica, L. M.; Stack, T. D. P.; Solomon, E. I. "Variable-Temperature Variable-Field Magnetic Circular Dichroism Studies of Tris-Hydroxy and μ_3-Oxo Bridged Trinuclear Cu(II) Complexes: Geometric and Electronic Structures of the Native Intermediate of Multicopper Oxidases." *J. Am. Chem. Soc.*, **2005**, *127*, 13680-13693.

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Diamine Ligand and Its Tyrosinase-like Reactivity." *J. Am. Chem. Soc.* **2002**, *124*, 9332-9333.

INVITED TALKS

15. "Late Transition Metal Catalysts for the Activation of Small Molecules: Relevance to Renewable Energy Catalysis." Department of Chemistry, *Macalester College*, September 2011.
14. "Late Transition Metal Catalysts for the Activation of Small Molecules: Relevance to Renewable Energy Catalysis." Department of Chemistry, *Western Michigan University*, September 2011.
13. "Late Transition Metal Catalysts for the Activation of Small Molecules: Relevance to Renewable Energy Catalysis." Challenges in Renewable Energy – International Symposia on Advancing the Chemical Sciences (ISACS4), *MIT, Boston*, July 2011.
12. "New Chemical Agents for Controlling Histone Demethylation and Amyloid β Peptide Aggregation." Department of Biochemistry and Molecular Biophysics, *Washington University School of Medicine*, April 2011.
11. "Late Transition Metal Catalysts for the Activation of Small Molecules: Relevance to Renewable Energy Catalysis." Department of Chemistry, *Saint Louis University*, February 2011.
10. "Late Transition Metal Catalysts for the Activation of Small Molecules: Relevance to Renewable Energy Catalysis." Department of Chemistry, departmental seminar and recruiting visit, *Southern Illinois University - Edwardsville*, January 2011.
9. "New Chemical Agents for Controlling Amyloid β Peptide Aggregation in Alzheimer's Disease." Alzheimer's Disease Research Center, *Washington University School of Medicine*, December 2010.
8. "Late Transition Metal Catalysts for the Activation of Small Molecules: Relevance to Renewable Energy Catalysis." Midstates Consortium Undergraduate Research Symposium, *Washington University in St. Louis*, November 2010.
7. "Stable Mononuclear Organometallic Pd(III) Complexes and Their C-C Bond Formation Reactivity." *Missouri Inorganic Day*, Saint Louis University, May 2010.
6. "Renewable Energy Catalysis: Studies of Water Oxidation by Bimetallic Complexes." Department of Chemistry and Biochemistry, *University of Missouri – St. Louis*, April 2010.
5. "New Chemical Agents for Imaging and Controlling Amyloid β Peptide Aggregation in Alzheimer's Disease." Department of Radiology, *Washington University School of Medicine*, September 2009.
4. "Renewable Energy Catalysis: Studies of Water Oxidation by Bimetallic Complexes." Departmental seminar and recruiting visit, *Illinois State University*, April 2009.

3. "Mechanistic Studies of the Ethylene-forming Enzyme ACC Oxidase." Invited Oral Presentation, *13th International Conference on Biological Inorganic Chemistry*, Vienna, Austria, July 2007.

2. "Tyrosinase Reactivity in a Model Complex: An Alternative Hydroxylation Mechanism." Invited Lecture, Young Investigator Symposium, *232nd National Meeting of the American Chemical Society*, San Francisco, September 2006.

1. "Phenolate Hydroxylation Reactivity of a μ-η^2:η^2-Peroxodicopper(II) Complex: Peroxide O–O Bond Cleavage Precedes C–O Bond Formation" Invited Lecture, *Gordon Graduate Research Seminar: Bioinorganic Chemistry*, Ventura, January 2005.

PRESENTATIONS and CONFERENCES

12. "New Chemical Agents for Controlling Amyloid β Peptide Aggregation in Alzheimer's Disease." Oral presentation, *International Conference on Biological Inorganic Chemistry (ICBIC15)*, Vancouver, August 2011.

11. *"*Stable Mononuclear Organometallic Pd(III) Complexes and Their C-C Bond Formation Reactivity.*"* Oral presentation and session chair, *American Chemical Society National Meeting*, Boston, August 2010.

10. *"*Stable Mononuclear Organometallic Pd(III) Complexes and Their C-C Bond Formation Reactivity.*"* Poster talk, *Organometallics Gordon Research Conference*, July 2010.

9. *"*Stable Mononuclear Organometallic Pd(III) Complexes and Their C-C Bond Formation Reactivity.*"* Poster talk, *Inorganic Chemistry Gordon Research Conference*, June 2010.

8. *"*Stable Mononuclear Organometallic Pd(III) Complexes and Their C-C Bond Formation Reactivity.*"* Poster presentation, *Department of Energy, Chemical Catalysis Meeting*, June 2010.

7. *"*Development of Specific Inhibitors for Histone Demethylases*"* Poster presentation and session chair, *Enzymes, Coenzymes & Metabolic Pathways Gordon Research Conference*, Waterville Valley, July 2009.

6. "Renewable Energy Catalysis: Studies of Water Oxidation by Binuclear Metal Complexes." Poster presentation, *Renewable Energy: Solar Fuels Gordon Research Conference*, Ventura, February 2009.

5. "The Nature of O_2 Activation by the Ethylene-Forming Enzyme ACC Oxidase." Poster presentation, *Metals in Biology Gordon Research Conference*, Ventura, January 2008.

4. "Interconversion of μ-η^2:η^2-Peroxodicopper(II) and Bis(μ-oxo)dicopper(III) Complexes: A Theoretical Study." Poster presentation, *12th International Conference on Biological Inorganic Chemistry*, Ann Arbor, Michigan, August 2005.

3. "Investigation of Tyrosinase-like Reactivity for a Cu/O_2 complex: Insights into the Phenol Hydroxylation Mechanism." Oral presentation, *227th National Meeting of the American Chemical Society*, Anaheim, March 2004.

2. "Detection and Characterization of Intermediates during the Hydroxylation of Phenols by a μ-η^2:η^2-Peroxodicopper(II) Complex." Poster presentation, *Gordon Graduate Research Seminar: Bioinorganic Chemistry*, Ventura, January 2004.

1. "Synthesis, Characterization, and Reactivity of a New μ-η^2:η^2-Peroxodicopper(II) Complex." Oral presentation, *224th National Meeting of the American Chemical Society*, Boston, August 2002.

SYNERGISTIC ACTIVITIES

- Reviewer for *Journal of the American Chemical Society*, *Proceedings of the National Academy of Sciences U.S.A., Inorganic Chemistry*, *Chemistry of Materials*, and *Dalton Transactions*.
- Grant proposal reviewer for *The Research Corporation, National Science Foundation, ACS Petroleum Research Fund*, and *The Alzheimer's Association*.